35

Art 34

CLAIMS

1. A binding assay process in which the concentration of an analyte in a liquid sample is determined by comparison with a dose-response curve computed from standard samples,

using a capture binding agent having binding sites specific for the analyte and a developing binding material capable of binding with the bound analyte or with the binding sites on the capture binding agent occupied by the bound analyte or with the binding sites remaining unoccupied on the binding agent,

the capture binding agent being used a small amount such that the capture binding agent has at most only an insignificant effect on the concentration of the analyte in the sample, the capture binding agent being immobilised at high density on a solid support, and

a label being used in the assay in relation to the developing binding material whereby the strength of the signal associated with the label is representative of the fractional occupancy of the binding sites on the capture binding agent by the analyte,

wherein there is used as the label microspheres having a size of less than 5μm and carrying a marker.

2. A process according to claim 1 wherein the small amount of capture binding agent binds less than about 5% of the analyte in the sample.

Elkins et al. 435/6
08/211,800
fd 6-22-94

 Art. 34

3. A process according to claim 1 ~~or claim 2~~ wherein the small amount of capture binding agent is immobilised at a surface density in the range of 1000 to 100,000 molecules /μm^2.

4. A process according to claim 1 ~~any one of the preceding claims~~ wherein the capture binding agent is immobilised on a solid support in the form of one or more microspots having an area of $1mm^2$ or less and wherein the liquid sample size is 1ml or less.

5. A process according to claim 4 wherein the microspot or microspots have a diameter of 0.01 to 1mm and contain immobilised capture binding agent at a surface density of 10,000 to 50,000 molecules/μm^2, the sample size being 50µl-1 ml.

6. A process according to claim 1 ~~any one of the preceding claims~~ wherein different capture binding agents are immobilised on different microspots on the same solid support and different binding assays for the determination of different analytes in the same liquid sample are performed in the same operation.

7. A process according to claim 1 ~~any one of the preceding claims~~ wherein the microspheres have a uniform size of 0.01 to 0.5µm.

Art.34

8. A process according to claim 1 ~~any one of the preceding claims~~ wherein the microspheres are made of polymer latex and are provided on their surface with negatively charged or positively charged groups.

9. A process according to claim 1 ~~any one of the preceding claims~~ wherein the marker is a fluorescent label contained within the microspheres.

10. A process according to claim 9 wherein the microspheres contain molecules of an oil-soluble fluorescent dye providing fluorescence in a colour range compatible with a standard filter set.

11. A process according to claim 9 ~~or claim 10~~ wherein the microspheres contain molecules of a fluorescent dye having a prolonged fluorescent period such that the signal strength is capable of being determined by a time-resolved fluorescence technique.

12. A process according to claim 1 ~~any one of the preceding claims~~ wherein the microspheres have the developing binding material adsorbed or directly or indirectly chemically bonded to them.

13. A process according to claim 1 ~~any one of the preceding claims~~ wherein the capture binding agent is adsorbed or directly or indirectly chemically bonded to microspheres, the

 Art. 34

microspheres being immobilised on the solid support and containing a different label from the label contained in the microspheres to which the developing binding material is adsorbed or chemically bonded.

14. A process according to claim 1 ~~any one of the preceding claims~~ wherein after the developing binding agent have been linked to the microspheres by adsorption or covalent bonding, the microspheres are blocked to avoid their non-specific binding to other materials.

15. A processing according to claim 14 wherein the blocking of the microspheres is achieved by means of bovine serum albumin or other non-interfering protein material and a non-ionic detergent.

16. A process according to claim 1 ~~any one of the preceding claims~~ wherein both the capture binding agent and the developing binding material are antibodies.

17. A process according to ~~any one of~~ claim 1 ~~to 15~~, for use in DNA assays, wherein the capture binding agent is single-stranded oligonucleotide DNA probe recognising a corresponding DNA sequence in the liquid sample and the developing binding material either is an antibody recognising only twin-stranded DNA sequences or is an oligonucleotide DNA sequence with either recognises another part of the corresponding DNA sequence in the liquid sample

 Art.34

or recognises residual single-stranded oligonucleotide DNA probe forming the capture binding agent, the developing binding material being labelled by means of the microspheres.

18. A process according to claim 1, wherein the binding assay is a non-competitive binding assay.

19. A binding assay process for the detection of an analyte comprising a single-stranded DNA sequence in a liquid sample, comprising

contacting the sample in a non-competitive or competitive procedure with

an immobilised capture binding agent which is a single-stranded oligonucleotide DNA probe capable of recognising analyte in the liquid sample and binding therewith, the capture binding agent being used a small amount such that the capture binding agent has at most only an insignificant effect on the concentration of the analyte in the sample, the capture binding agent being immobilised at high density on a solid support, and

with a labelled developing binding material which either is an antibody capable of recognising only twin-stranded DNA sequences formed from the probe and the analyte and of binding therewith or is an oligonucleotide DNA sequence capable of recognising and binding with either another part of the analyte or the residual probe,

the developing binding material being labelled by means of microspheres having a size of less than 5µm and carrying a marker, and,

after the removal of unattached developing binding material, detecting the presence of the analyte by the existence or strength of a signal from the marker attached to developing binding material which has become bonded directly or indirectly to the immobilised capture binding agent.

20. A process according to claim 19, wherein the marker is a fluorescent label contained within microspheres having a size of 0.01 to 1µm.

21. A process according to claim 19 ~~or claim 20~~, wherein the developing binding material is directly or indirectly covalently bonded to the microspheres.

22. A kit for use in a binding assay process in which the concentration of an analyte in a liquid sample is determined,

using a capture binding agent having binding site-specific for the analyte and a developing binding material capable of binding with the bound analyte or with the binding sites on the capture binding agent occupied by the bound analyte or with the binding sites remaining unoccupied on the capture binding agent,

the capture binding agent being used a small amount

such that the capture binding agent has at most only an insignificant effect on the concentration of the analyte in the sample, the capture binding agent being immobilised at high density on a solid support,

a label being used in relation to the developing binding material whereby the strength of the signal associated with the label is representative of the fractional occupancy of the binding sites on the capture binding agent by the analyte,

the kit comprising (a) a solid support having the capture binding agent immobilised at high density thereon, (b) a developing reagent comprising the developing binding material adsorbed or directly or indirectly chemically bonded to the surface of microspheres carrying a marker and (c) standards having known amounts or concentrations of the analyte to be determined.

23. A kit according to claim 22 wherein the small amount of capture binding agent binds less than about 5% of the analyte in the sample.

24. A kit according to claim 22 ~~or claim 23~~ wherein the solid support has the capture binding agent immobilised thereon in the form of one or more microspots of size less than 1mm² and surface density of at least 1000 molecules/μm².

25. A kit according to claim ~~any one of claims~~ 22 ~~to 24~~ wherein

Art. 34

different capture binding agents are immobilised on different microspots on the same solid support and a plurality of different developing reagents and different standards for different analytes are included.

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